5.—INVESTIGATIONS ON THE "LEAF SPOT" DISEASE OF BLACK MULBERRIES CAUSED BY *SEPTOGLOEUM MORI* (Briosi and Cavara).

by

R. E. STEWART, B.Sc.

(Botany Department, University of W.A.)

Read 8th June, 1948.

SUMMARY.

1. A new disease of Mulberry trees is described. The fungus responsible has been isolated and identified as *Septogloeum mori*.
2. The pathogenicity of the fungus has been demonstrated by inoculation experiments.
3. The general morphology, spore types and some of the cultural characters of the parasite are described.
4. Host-parasite relationships have been investigated, attention being given to histopathology, spore dissemination, overwintering of *S. mori*, and the distribution of the disease.
5. The disease has been successfully controlled by spraying either with Lime sulphur or with Bordeaux Mixture. Details of spray programmes are given.

INTRODUCTION.

In Western Australia, the Black Mulberry (*Morus nigra*) was for many years affected by one serious disease only, namely "Bacterial Blight" caused by *Bacterium mori*, (Boyer and Lambert).

In 1943, however, a fungal "leaf spot" disease was also recorded (1). At first, this disease appeared to be of minor importance, but in recent years, it has spread so rapidly that it is now found in most areas of the State where mulberries are grown, and threatens to supersede the "Bacterial Blight" in importance.

The mulberry is of small commercial value in W.A., being grown mainly as single trees in home gardens and orchards ; however, it is much prized as a source of fresh fruit. The following investigation was therefore undertaken early in 1946, to determine the cause of the disease, its method of carry-over, and if possible to confirm measures recommended by the Government Plant Pathologist for its control.

SYMPTOMS OF THE DISEASE.

The disease is confined to the leaves, where it causes regular necrotic areas (1–10 mm. in diameter), with dark brown margins and typically white centres. The area of necrosis is usually surrounded by a chlorotic margin of greater or less extent. Veins immediately surrounding the necrotic area tend to become brown. In the early part of the season, the spot is very small (1–2 mm.) and almost black in colour. The chlorotic region is also small. As the season advances, the spots grow irregularly in area. Each new growth region has its own permanent dark margin, so that the necrotic area eventually becomes marked by a wave-like pattern. (See Plate I.). At the same time, the centre of the spot becomes white, and this white area tends to spread outwards as the necrotic area increases.

The spots may be small and very numerous, or very large, but few in number. In either case they tend to coalesce and kill large areas of leaf tissue.

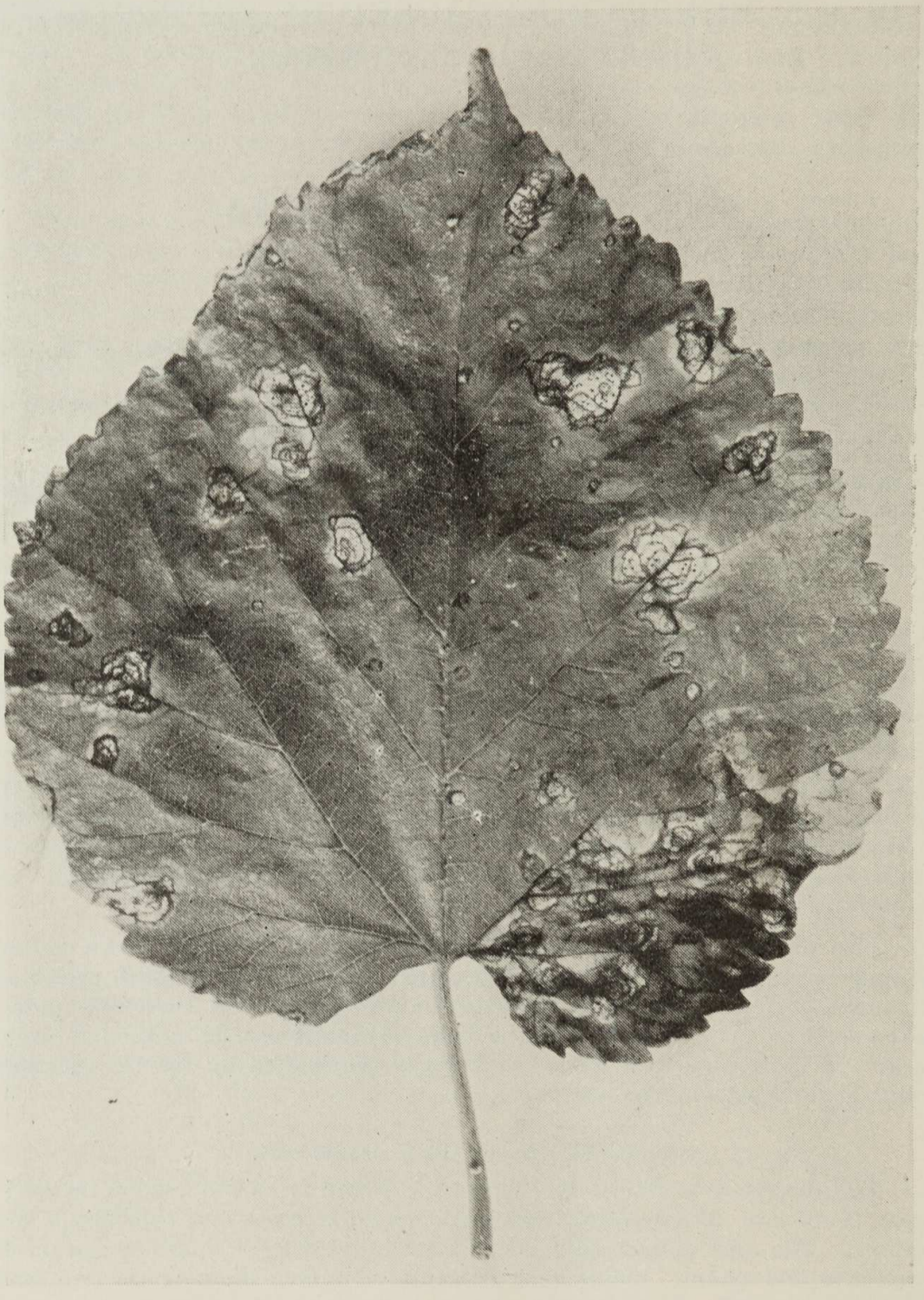


Plate I.

Mulberry leaf showing the "leaf spot" symptoms caused by *Septogloeum mori*. Photograph from *Journ. Dept. Agric. W. Aust.*, Vol. XXIV. (Second Series) No. 1, 1947, by courtesy of Department of Agriculture, W.A.

The acervuli appear first as fine black spots in the white centre of the infected area. Later when the epidermis has erupted, the spore pustules show a faint pink colour.

The disease causes premature defoliation leading to dropping of fruit. In a bad season early defoliation results in almost complete loss of the crop.

FUNGUS ASSOCIATED WITH THE SYMPTOMS.

The fungus found to be constantly associated with the above symptoms, was identified as *Septogloeum mori* (Briosi and Cavara). (4) (15).

Initial cultures of the organismn were made by plating out portions of infected leaf material, surface sterilized with mercuric chloride 1:1500 for 1¼ min. ; and washed well with sterile distilled water.

Septogloeum came up constantly in the plates. A number of other fungi occurred less consistently. These included, *Fusarium* sp., *Alternaria* sp., *Chaetomium* sp., *Penicillium* sp., *Verticillium* sp., *Amaurascus* sp., *Phoma* sp., and *Phomopsis* sp. Yeasts and bacteria were sometimes present.

When surface sterilization with calcium hypochlorite was practised, only *Fusarium* sp., and *Alternaria* sp., were occasionally present with the consistently occurring *Septogloeum*.

Phleospora mori (=*Septogleoum mori*) was recorded by McAlpine (7) as the cause of a mulberry leaf spot in Victoria and Tasmania. It is also known to occur in South Australia, Europe (4), North America (4), Central Asia (14), and Belgian Congo (5).

Septogloeum mori has been found to attack white (*M. alba*) (12), red (*M. rubra*) (13), and black (*M. nigra*) (12) mulberries.

DESCRIPTION OF THE FUNGUS.

The Acervulus.

The fruiting body is in the form of an erumpent type of acervulus 1–2 mm. in diameter. The stroma is of compacted dark mycelium, 20 μ or more thick. Sporophores are erect, approximately 30 μ in length and vary from olive brown to hyaline. Spores of two types were found to be present.

Those on living leaves were hyaline, cylindrical, curvulus, 1–5 septate (rarely 0 or 5) guttulate, dimensions 17 μ–53 μ x 4.5 μ borne singly.

On dead and dying leaves, spores were dark to olive brown, cylindrical, elongate, torulose 1–9 septate, guttulate forms, 20–70 μ x 5 μ, borne singly.

Study of the hyaline spore type led to the organism being identified as *Septogloeum mori* (2) (4), belonging to the FUNGI IMPERFECTI.

The synonymy for *Septogloeum mori* is as follows :—

Septoria mori Lev. (4) (13)
Septoria moricola Pass. (14)
Fusarium maculans Bereng. (4)
Phleospora mori Sacc. (4) (6) (13)
Phleospora moricola (Pass.) Sacc. (4) (13) (14)
Phleospora maculans All. (4) (15)
Cylindrosporium mori Berl. (3) (11)
Cylindrosporium moricola (14)
Cylindrosporium maculans All. (15)

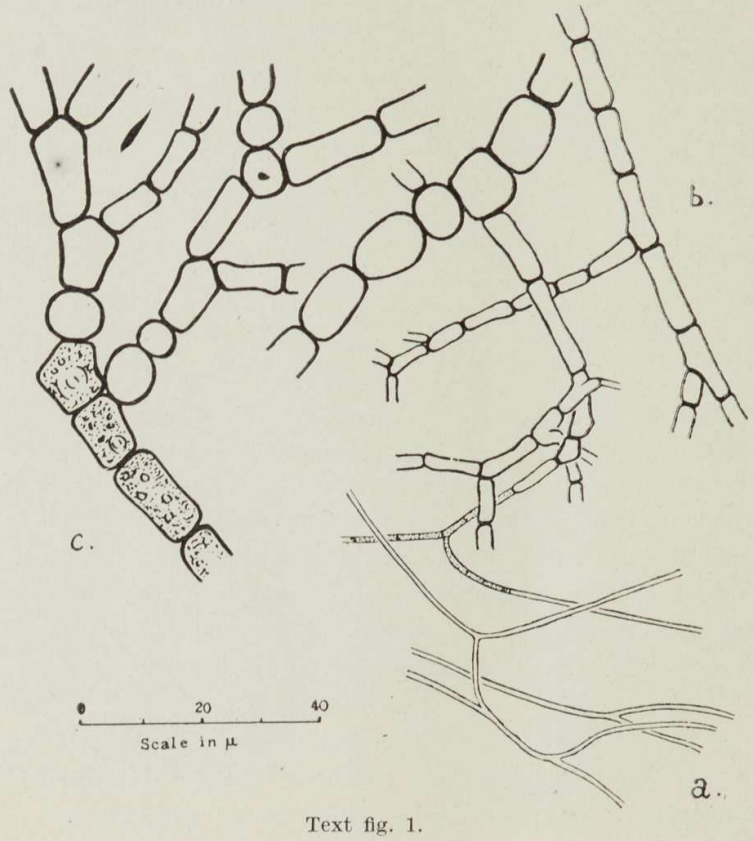
No reference can be found in the literature to the "dark" spore form referred to above. The perfect stage of *Septogloeum* appears still to be a matter for debate. Stevens (11) considers it to be *Mycosphaerella morifolia*, while Ivanoff (5) and Wolf (13) have recorded it as *M. mori*. No confirmatory evidence of either could be found in the course of this work.

The extent of the initial infection of *S. mori* in the mulberry leaf is relatively small, and the invading hyphae sparse, until just prior to spore formation when the mycelium rapidly proliferates beneath the epidermis to form the mass of the stroma.

At this stage, the mycelium exhibits two types of hyphae :—

(a) Fine hyaline hyphae 1–2 μ in diameter, containing a granular protoplast (See text fig. 1a.). This type constitutes the feeding mycelium in the mulberry leaf. In culture, it forms as well a mass of aerial hyphae which completely covers the whole colony, and at times may take on a pinkish to mauve hue.

(b) Thick walled, olive brown mycelium 4–12 μ in diameter generally containing a granular protoplast with highly refractive globules, and large central vacuole.



Text fig. 1.
a. Fine hyaline hyphae.
b. and c. Thick walled dark hyphae.

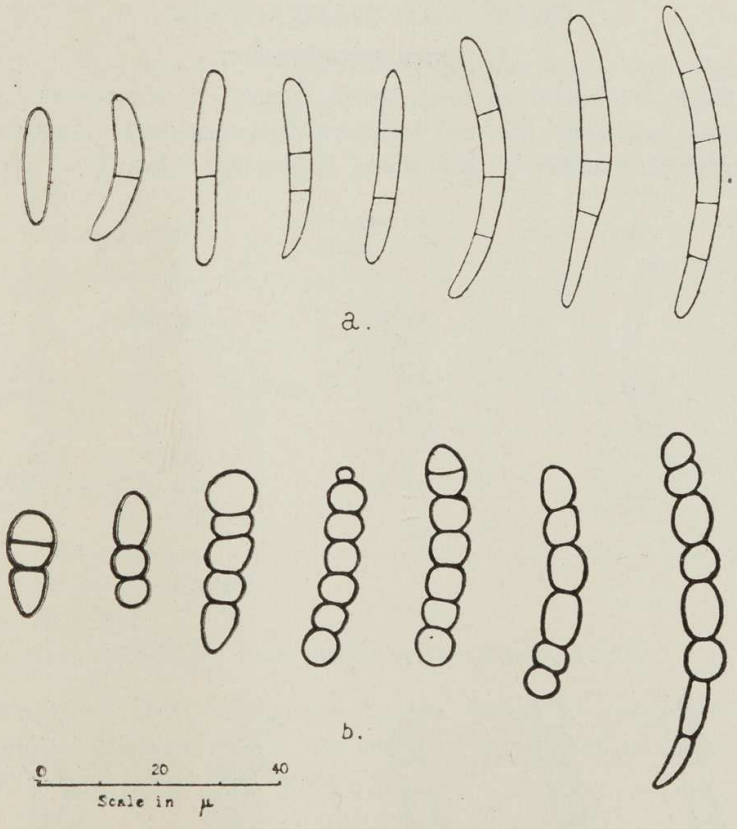
This thick walled mycelium can be separated into two types, one forming rather fine, paler, regular filaments (4 μ in diameter) (See text fig. 1b), the other forming more irregular hyphae, the cells of which tend to be short and globular (8 μ x 10 μ – 12 μ) (See text fig. 1c). In the mulberry leaf these two types or dark mycelium are incorporated in the acervulus. In culture, one or both types may form part of the ground mycelium as well, or in extreme cases, form practically the whole of the fungal body.

The acervulus is more or less circular in shape, but many frequently coalesce to give irregular shaped masses. The stroma of the acervulus is composed of the thickest dark mycelium. This dark mycelium tends to become knotted and packed together to form a pseudo-parenchymatous structure.

The sporophore "layer" is continuous over that surface of the stroma which is adjacent to the epidermis. Wolf (13) describes the sporophores as short, hyaline, 3–10 septate ; but this was not found to be the case here. Investigations of diseased leaf material showed the sporophores to have dark basal cells, which became lighter and thinner walled towards the apex. The apical cell of the sporophore was typically hyaline. The sporophores are erect, and closely packed, they vary in length from 10–40 μ or more, and bear the hyaline spores terminally.

As indicated previously, the spores of *S. mori* are of two types both of which are asexual (a fine hyaline spore, and a thick dark spore). Extreme forms of these spores are distinctly different, and are borne as two separate stages in the life history. The hyaline spores are produced on the living host, while the fungus is actively growing, and they serve to spread the disease during the growing season. These spores have been designated the "Summer" spores, and will be referred to by that name throughout the remainder of the text.

The second spore type (i.e., dark spores) are produced on dead leaves, and in the late autumn have been observed on leaves still attached to the tree. They may be regarded as overwintering forms. This spore type will be referred to as the "Autumn" spore type.



Text fig. 2.

a. Thin walled "Summer" spores.
b. Thick walled dark "Autumn" spores.

The description of the spore types is as follows :—

(*a*) "*Summer*" *Spores.*

Thin walled, hyaline, elongate, multi-septate (0–5) curvulus, guttulate forms approximately 33 μ x 4.5 μ. (See text fig. 2a).

These " Summer " spores are produced both naturally (i.e., on the living host), and in certain culture media (see Table 2.). Spore dimensions (measurements of the length of 100 spores from naturally infected mulberry leaves taken in April, 1946), gave a mean value of 33 μ, with a range from 17.5 μ to 52.5 μ. The breadth was more constant, averaging 4.5 μ. The number of septa was very variable, being from 0–5 ; the average being 2–3 septa.

(*b*) " *Autumn* " *Spores.*

Thick walled, dark to olive brown, cylindrical elongate, torulose, 1-9 septate, guttulate forms approximately 45 μ x 5 μ. (See text fig. 2b).

These "Autumn" spores occur on leaves in the late Autumn, and on dead infected leaf material. They have also been found to occur in dried out cultures which initially produced "Summer" spores.

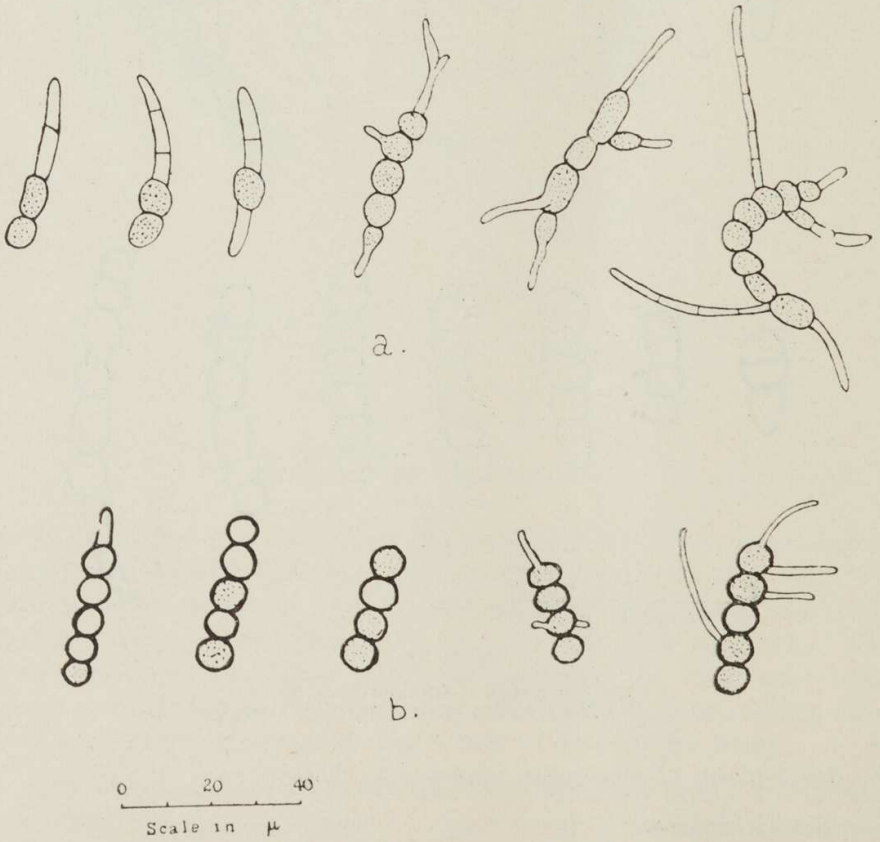
The dimensions of these spores show greater variability than the "Summer" spores. The range in length was from 20–70 μ, and in diameter from 4.5 μ to 5.5 μ. The number of septa varied from 1–9. At each septum, the spore tends to be constricted, giving each segment a more rounded appearance than the segments of the "Summer" spores. Typically, the "Autumn" spores are olive brown, but a range of colour to dark purplish grey has been observed.

The "Summer" spores are borne on young leaves but just prior to leaf fall, they begin to be replaced by "Autumn" spores.

CULTURAL CHARACTERS.

1. Spore germination.

Germination is similar in both spore types. Upon placing the spores in water, the spore segments fill out, and tend to round up. Just prior to germination they become densely granular. (See text fig. 3.). In both spore



Text fig. 3.

Germinating Spores.

a. " Summer " spores.
b. " Autumn " spores.

types the germ tubes, which may develop from one or more segments, are fine and hyaline. In "Summer" spores germinated in water the germ tubes were found to anastomose freely with germ tubes of the same or of different spores. Initial branching of germ tubes is not profuse.

Under suitable temperature conditions (20°–30°C), "Summer" spores germinated in distilled water within 24 hours. Difficulty was experienced in getting the "Autumn" spores to germinate in water, but they germinated freely after 36 hours in a mulberry leaf extract.

The optimum temperature for spore germination was found to be approximately 25°C, this being very close to that required for maximum vegetative growth. The maximum temperature for "Summer" spores was found to be 36°C; they were killed by 24 hours at 38°C. "Autumn" spores survived exposure for 36 hours at 42°C, indicating a higher degree of resistance. "Autumn" spores which had been subjected to winter temperature conditions were 80 per cent. viable when germinated at 24°C. "Summer" spores, kept under laboratory conditions over the winter, were less than 1 per cent viable.

The effect of temperature and type of medium on the germination of spores of *S. mori* is shown in Table 1.

TABLE 1.

Effect of Temperature and Type of Medium on Spore Germination.

Temperature °C.	Per cent. Germination. Summer Spores—24 hours.		Per cent. Germination. Autumn Spores—36 hours.	
	Agar Jelly.	Mulberry Leaf Extract Jelly.	Agar Jelly.	Mulberry Leaf Extract Jelly.
20°	95	98	....	....
24°	97	98	....	80
30°	80	95	....	....
34°	68	75	8	43
36°	0 (a)	0 (a)	0 (c)	0 (c)
38°	0 (b)	0 (b)	0 (c)	0 (c)
40°	0 (b)	0 (b)	0 (c)	0 (c)
42°	....	....	0 (c)	0 (c)

(a) Spores 95 per cent. killed.

(b) Spores all killed.

(c) Spores more than 50 per cent. viable, when retested at 25°C.

Both "Summer" and "Autumn" spores failed to germinate when submerged in agar, or in liquid which was not freely exposed to the air. This indicates their strong aerobic tendencies.

2. Cultures on Artificial Media-Physiology.

Preliminary experiments on the cultural habits of *S. mori* were conducted to give some indication of its requirements.

Considerable variation in the growth of the colony was observed, depending upon the type of media used, its pH value and the temperature of incubation.

Cultures on Potato Dextrose Agar (10) gave a small compact colony, more or less circular, pulvinate with smooth surface and entire margin. With increase in depth of the media, the surface of the colony tended to become rugose. Dark hyphae constituted the feeding mycelium, which was covered by a thin felt of fine white hyaline hyphae—no spores were produced, even when the colony was placed in the sunlight.

Cultural characters for other media used are contained in Table 2, while Temperature relationships are given in Table 3, and pH relationships in Table 4.

TABLE 2.

Media Relationships.

Media.	Growth (Diam. of colony) in 21 days.	Spore Production.	Remarks.
1. P.D.A.	12 mm.	−ve.	Small compact growth with fine aerial, and dark ground mycelium.
2. P.D.A. + 0.5 % 'Marmite'	14 mm.	−ve.	
3. 'Marmite' P.D.A. + 0.2% NH_4NO_3	29 mm.	+ve., spores of normal size *	Good healthy growth, with rather flat colony of white aerial and dark ground mycelium.
4. P.D.A. + 5% Mulberry leaf Extract †	....	−ve.	Small compact growth as 1 and 2, but with fine grey aerial mycelium, and dark ground mycelium.
5. 5% Mulberry leaf Extract Agar	12 mm.	−ve.	
6. Conn's Agar (8)	23 mm.	Spores a little less than normal in length	Healthy fluffy growth with excess of fine white aerial mycelium.
7. Conn's Agar + 0.5% 'Marmite'	24 mm.	Spores a little longer than normal	Growth a little more dense than with Conn's Agar, also has excess of fine white mycelium
8. Conn's Agar + 1% Mulberry leaf Extract	23 mm.	Spore size normal	Much darker denser growth than 6 and 7, with great quantity of dark ground mycelium and greyish aerial mycelium.
9. Conn's Agar + 2% Mulberry leaf Extract	25 mm.	Spore size normal	
10. Conn's Agar + 4% Mulberry leaf Extract	25 mm.	Spore size normal	
11. Shear's Corn Meal Agar (9)	15 mm.	−ve.	Small compact growth with fine white aerial and dark ground mycelium
12. Standard Agar (8)	17.5 mm.	−ve.	Small more sparse growth with practically no dark mycelium
13. Leonian's Agar (9)	6 mm.	−ve.	Very restricted compact growth, with fine aerial and dark ground mycelium

* Normal spore length was taken as the average length produced naturally—i.e., 33μ x 4.5μ.

† "A 5 per cent. mulberry leaf extract was made by boiling 50 gms. of dried ground leaf material with water for 20 minutes ; this was filtered and made up with water to 1 litre. When combined with other media, this extract was used in place of water."

TABLE 3.

Temperature Relationships.

(Conn's Agar used as standard media, pH adjusted to 4.5).

Temperature.	Growth (Diam. of Colony) in 16 days.	Remarks.
10°C	0.0 mm.	No growth, but colony not killed.
22°C	21.9 mm.	Good growth, production of spores within 12 days.
30°C	12.0 mm.	Only white growth ; no spores produced.
35°C	0.0 mm	No growth, culture not killed.
40°C	0.0 mm	Killed within 48 hours at this temperature.

The above table indicates that temperature has a marked effect both on mycelial growth and on spore production.

TABLE 4.

pH Relationships.

(Using Conn's Agar at 22°C.)

pH of Media.	Growth (Diameter of Colony) in 24 days.	Remarks.
4.2	24.0 mm.	Culture healthy with abundant fine white mycelium. Good spore production.
5.0	28.7 mm.	Culture healthy with characteristic ragged edges. Very good spore production.
6.0	29.9 mm.	As for pH5, but spores slightly larger.
7.0	20.1 mm.	Dark ground mycelium in excess of light aerial. Spore production small. Actual growth more sparse.
8.0	23.2 mm.	As for pH7, but actual growth much more sparse. Spore production occurs much later, and is less abundant than for pH ranges between 4.2 and 8.0.
9.0	22.1 mm.	

This table indicates that a pH between 5 and 6 is the most suitable. It may be noted that *S. mori* grows well both in very acid and in very alkaline media.

3. Spore Production in Culture.

"Summer" spores produced in culture were found to be morphologically almost identical with those produced naturally, although some difference in size was observed.

The connection between spore types was ascertained. In older cultures which had been taken from a single hyaline spore culture, a range of types from the hyaline "Summer" spore to the dark "Autumn" spore was observed. (See text fig. 4.).

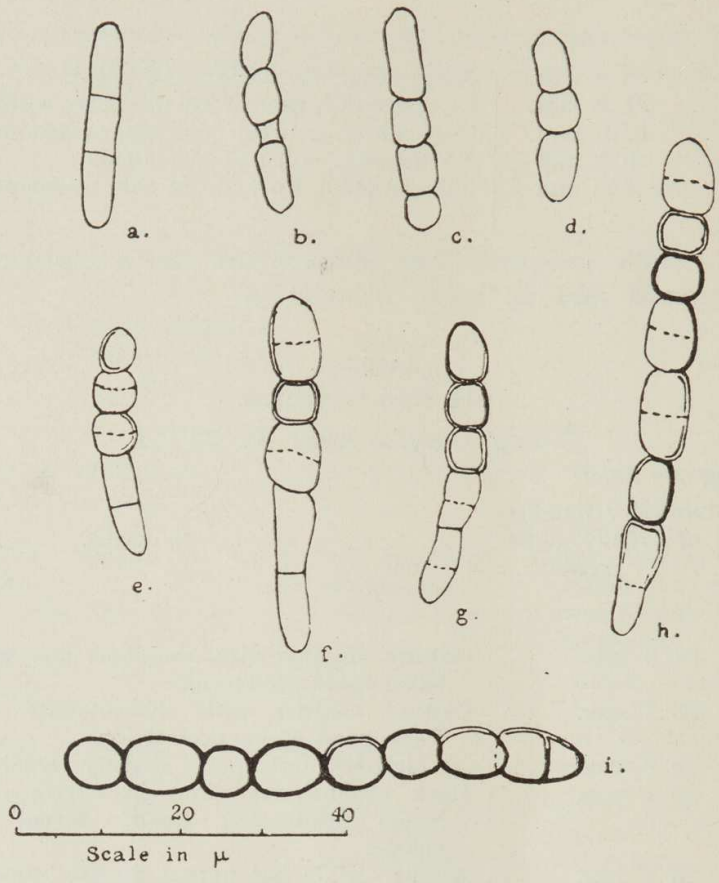
MODE OF INFECTION.

The relationship between host and parasite was investigated by sectioning both naturally and artificially infected material.

In microtomed sections of mulberry leaf material, that had been artificially inoculated for 28 hours before fixing, germ tubes of *S. mori* were observed entering the stomates. No indication of cuticular penetration was found.

Inoculation, in the field, of leaves which were kept under humid conditions for 28 hours, showed, upon examination, germ tubes entering the stomates.

Further experiments in the laboratory, confirmed the results in the field. Stomata are found on the lower surface of the leaf only, and infection occurred only on leaves inoculated on the lower surface.



Text fig. 4.
Stages in the formation of "Autumn" spores of *Septogloeum mori*.

HISTO-PATHOLOGY.

Using microtomed sections, the course of infection was traced, and found to be as follows :—

After the germ tube penetrates the host, the mycelium first invades the spongy mesophyll. Its growth is mostly intercellular and in course of time the fungus spreads through most of the host tissue. It invades particularly the parenchyma sheath of the vascular bundle. (See text fig. 5).

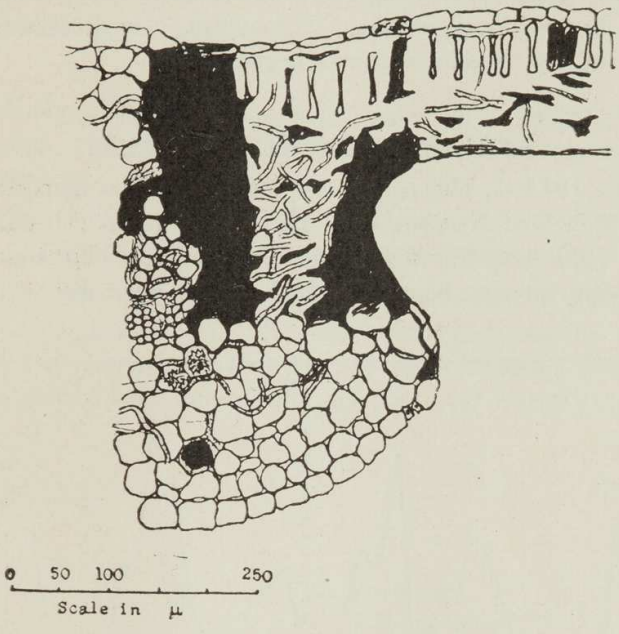
Throughout the whole of this vegetative growth, the mycelial strands are quite sparse. They extend well beyond the limits of the necrotic spot, possibly to the extent of the chlorotic area.

About the tenth day after infection, the mycelium in the centre of the diseased region tends to anastomose beneath the epidermis to form the acervulus. Acervuli occur commonly on the upper surface of the leaf, but they may be formed on the lower surface also.

This mass of anastomosing tissue replaces the inner and side walls of the epidermal cells, while the outer wall and cuticle remain intact. From this mass of mycelium, the acervulus is formed, and it produces spores irrespective of the external moisture conditions.

Several acervuli are produced within each "spot."

Late in Autumn the infected areas bear the "Autumn" spores. The "Autumn" spores borne on the acervulus of the dead leaf form a very compact mass, which tends to remain as such, and they are only released with the decomposition of the leaf.



Text fig. 5.
T. S. of mulberry leaf showing the invasion of the vascular sheath by mycelium of *S. mori* and the production of tannin in the blackened areas.

SPORE DISSEMINATION.

By exposing glycerined slides, evidence was obtained that the "Summer" spores of *S. mori* were wind borne and that they were released only during rainy weather.

Given suitable moisture conditions, the epidermis above the acervulus blisters, and, with continued high humidity, bursts, exposing the faintly pink pustules of spores.

Dissemination of "Autumn" spores is also believed to be by wind. As the spores were found lodged in all conceivable crevices of the trees it seems unlikely that any other agency could distribute them so well. The "Autumn" spores are released by the decomposition of the leaf material.

OVERWINTERING OF *S. MORI*.

The overwintering of *S. mori* is carried out by the thick walled "Autumn" spores previously described.

A search was made for a sexual stage of the fungus. Infected leaves which had fallen naturally, were trapped beneath wire netting and allowed to decay in that condition, while other leaves were held in a damp condition in the laboratory.

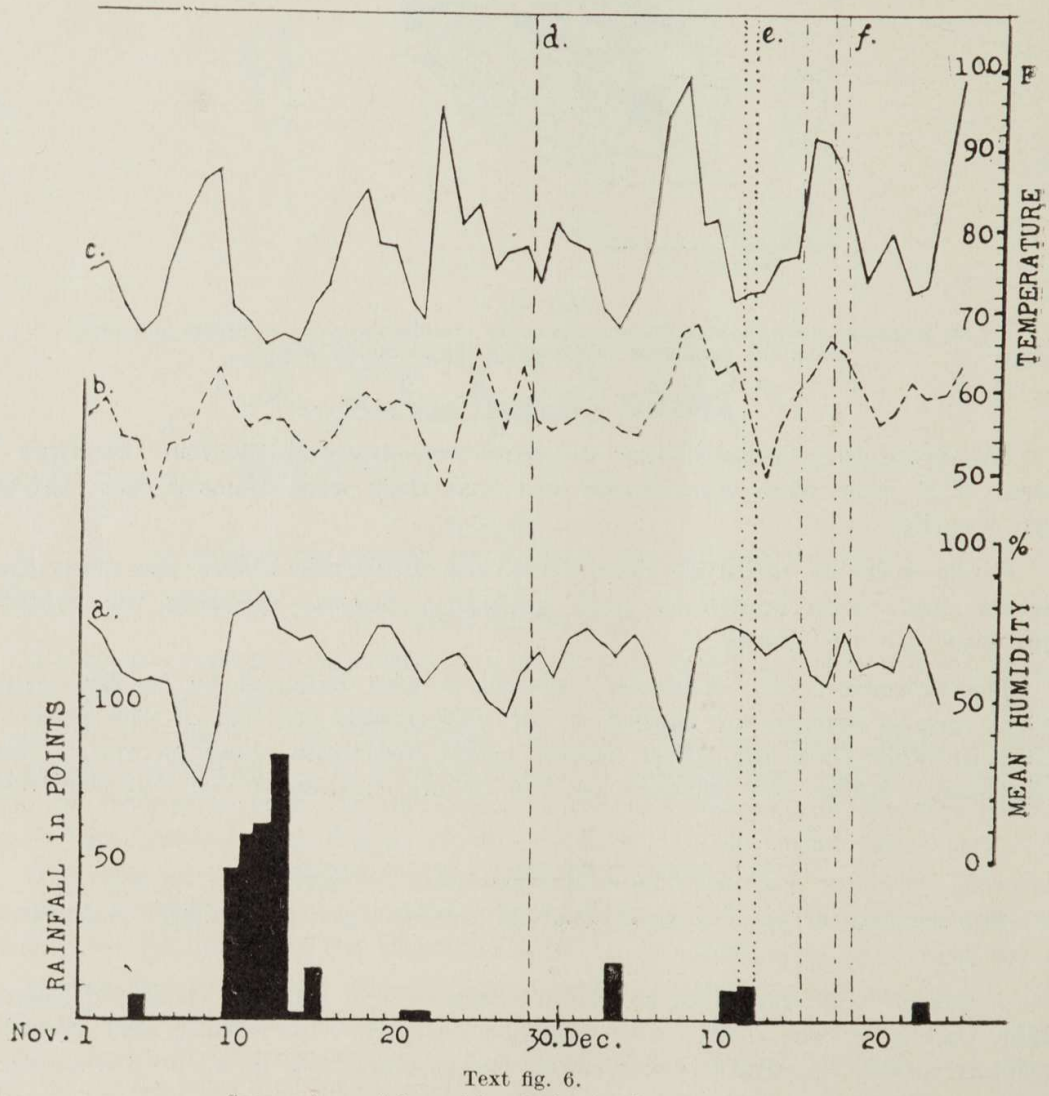
The fungal flora of the above leaves was investigated, paying particular attention to regions of infection. In these regions numerous pustules of "Autumn" spores (of *S. mori*) were consistently found. The fungi most commonly associated with them were species of *Phoma* and *Alternaria*.

Fungi of less frequent occurrence were species of *Fusarium*, *Phomopsis*, *Pleomassaria*, *Amaurascus*, and a slime mould. Neither *Pleomassaria* nor *Amaurascus* could be shown to be related to *Septogloeum mori*, as both produced only their sexual stage in culture. There was no other sexual form found, and no trace of the sexual stage of *S. mori* in dead leaf material.

In a further search for the sexual phase of *S. mori* dead twigs and wood, lenticels (especially those diseased), wood cankers, buds and leaf scars were closely examined but without result. " Autumn " spores only were found deposited in most of the regions investigated.

COURSE OF THE DISEASE.

In the season, 1946–7, the mulberry leaf spot first appeared in the metropolitan area at the end of November. Extensive rains fell early in November, accompanied by cool weather and high humidity. Eighteen days after the first fall of rain the disease was recorded. (See text fig. 6). This outbreak



Text fig. 6.

Spore release of *S. mori* in relation to climatic conditions

a. Mean humidity.
b. Minimum Temperature.
c. Maximum Temperature.
d. First appearance of natural infection of *S. mori*.
e. Times of natural release of spores of *S. mori*.
f. Times when no release (natural) could be detected.

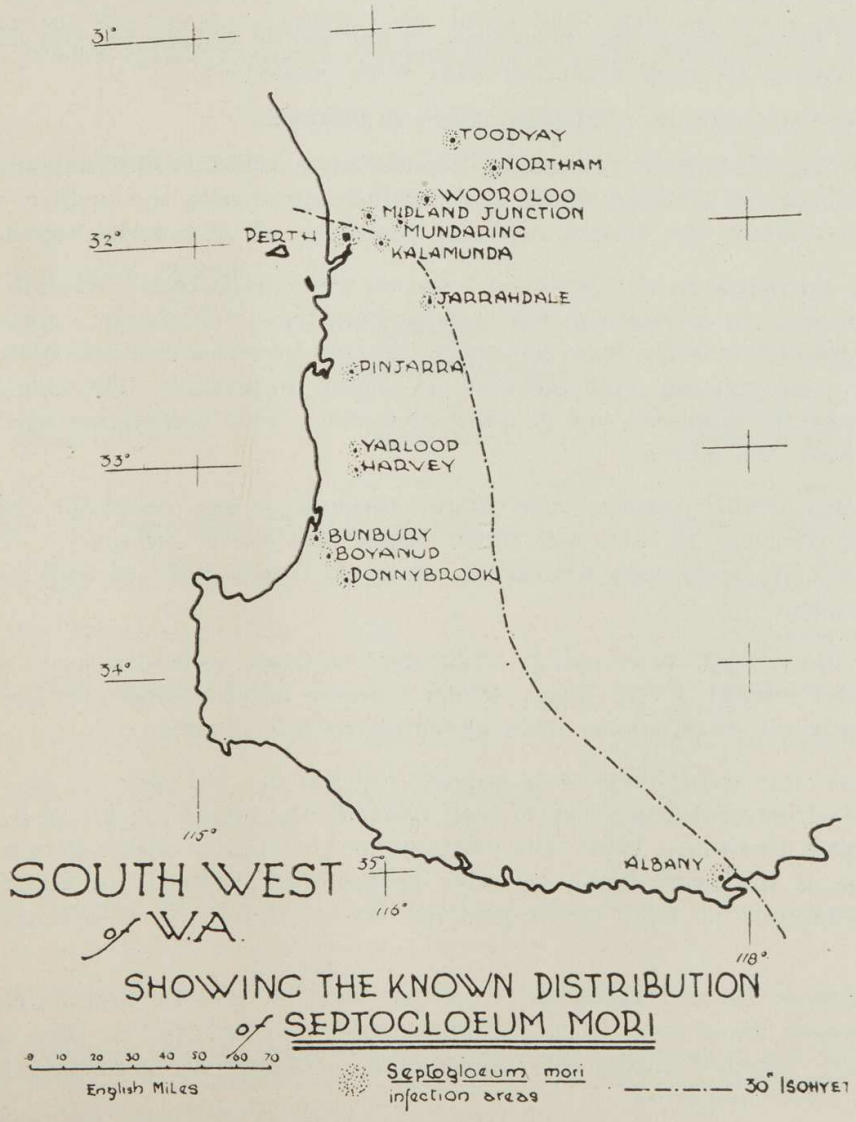
was extensive throughout the metropolitan area, none of the trees under observation escaping. Further rains fell early in December, and by Christmass the trees were nearly as badly infected as they had been at the end of 1945–46 season when this investigation was commenced. Scarcely any leaves were left without spots, and large areas of many infected leaves were killed.

By early January, many of the control trees were practically bare of foliage. Others were not so badly affected, but even there, de-foliation had commenced.

The onset of the disease appears to have been suspended over the hot, dry January period, the condition of the trees remaining much the same as they were in December.

DISTRIBUTION OF THE DISEASE.

In the South West, the disease has been recorded in early summer, occurring before fruit ripening stage. It has a fairly extensive distribution, (see text fig. 7), mainly occurring in the 30 in. to 40 in. rainfall area, where temperatures during November and December are low and humidity high.



Text Fig. 7.

The most northerly regions in which the disease has been recorded are Toodyay and Northam. Both these towns lie well within the 20 in. to 30 in. rainfall belt, but being situated on a river have slightly higher humidity than the surrounding districts.

This distribution suggests that it is a disease of cooler, more humid regions. It may be noted that this disease also occurs in France under moist conditions (3).

INOCULATION EXPERIMENTS.

After isolation of the organism, inoculation experiments were carried out to test for pathogenicity.

Mature trees in the field together with three-year old trees kept under glass house conditions were used for the experiments.

Technique.

Sterile bottles were placed over test leaves, the necks protected with cotton wool, and the bottles supported to prevent strain on leaf or stalk.

Leaves selected were well shaded, so as to avoid direct rays of the sun.

The leaves under test were kept in the sterile bottles for ten days prior to inoculation to make sure that they were healthy.

Spore suspensions used were made as follows :—

(a) Suspensions of " Summer " spores were made by flooding mature cultures with sterile distilled water, and carefully disturbing the surface mycelium so as to release the spores, without breaking off the aerial mycelium.

(b) Suspensions of " Autumn " spores were made from naturally infected leaf material, as no culture was entirely free from " Summer " spores. This leaf material was taken from naturally infected leaves which had been trapped beneath wire-netting and allowed to decay naturally. The spore masses were carefully dissected out in distilled water. This suspension was agitated to separate the spores.

Using sterile pipettes, the spore suspension was carefully dropped on mapped portions of each leaf (both upper and lower surfaces). The rough nature of the epidermis allowed a relatively large drop of inoculum to remain in situ.

In inoculation tests using " Summer " spores, typical disease symptoms developed within 10–14 days, while control leaves remained healthy. In practically all cases, spores were produced within 12 days.

A similar experiment was carried out on an old tree in the field, the inoculated leaves being situated well towards the centre of the tree, to shade them from the sun. With this experiment the results obtained were similar to those of the small trees, but the disease was produced in a shorter time. This difference in time could perhaps be attributed to temperature differences.

In tests using " Autumn " spores, positive results were obtained, but the diseased areas took twenty (20) days to produce spores. The occurrence of hot dry weather during this experiment is believed to be responsible for the long incubation period.

THE COURSE OF SYMPTOMS FOLLOWING ARTIFICIAL INOCULATION.

The first visible signs of infection following artificial inoculation occurred between the fifth and eighth days when chlorotic areas with diffuse margins became apparent. This was followed within the next two days by the appearance of a slightly discoloured spot in the centre of the chlorotic area. This spot enlarged and darkened until by the 10–14th day, it was dark brown in colour, 2–3mm. in diameter. It forms the original necrotic area. If weather conditions are suitable once this stage has been reached, the epidermis erupts and the spores are exposed.

Further growth of the spot is slow. It retains the halo of chlorotic tissue, while the centre of the spot becomes white, (the whole of the internal leaf tissue is disintegrated and partially absorbed by the parasite, so that only the upper and lower epidermis remain intact).

When the growth is temporarily suspended, the margin of the spot becomes much darker, becoming impregnated with tannin, which remains within the necrotic area when growth of the parasite proceeds.

The actual manifestation of the symptoms was found to vary with the environmental conditions. Infection under cool, moist conditions (on vigorously growing leaves) gave small dark spots with little chlorosis, while hot, moist conditions gave extensive chlorosis and a very pale spot.

CONTROL MEASURES.

In experiments designed to control this leaf spot disease, two sprays were tested :—

(a) Lime-sulphur.
(b) Bordeaux mixture.

TABLE 5.

Spray Schedule.

Tree No.	Time of Spray.	Spray Used.	Strength of Spray.
I.	At Bud Movement 28–9–46	Lime-sulphur	1/15 (Polysulphide content*= 1.44 %)
	After Bud Burst 14–10–46	do. do.	1/50 (Polysulphide content = 0.432 %)
II.	At Bud Movement 28–9–46	do. do.	1/15 (Polysulphide content = 1.44 %)
	After Bud Burst 14–10–46	do. do.	1/50 (Polysulphide content = 0.432 %)
	After Fruit Set 18–11–46	do. do.	1/100 (Polysulphide content = 0.216 %)
III.	At Bud Movement 28–9–46	Bordeaux mixture....	6 : 4 : 40.
	After Bud Burst 14–10–46	do. do.	3 : 4 : 40.
IV.†	At Bud Movement 28–9–46	do. do.	6 : 4 : 40.
	After Bud Burst 14–10–46	do. do.	3 : 4 : 40.
	After Fruit Set 18–11–46	do. do.	2 : 4 : 40.

In all cases calcium caseinate was used as a spreader at the rate of ½ lb. per 40 gallons of spray.

* Estimation of polysulphide content of the stock Lime-sulphur was kindly made by Mr. A. R. H. Cole (Research Officer, Department of Chemistry, University of W.A.).

† This tree was within 20 yards of an unsprayed control tree.

As there were only six trees in the same vicinity (i.e., Nedlands district) available for spraying, treatment was limited to four trees sprayed, two with Lime-sulphur, and two with Bordeaux mixture, while two trees were kept as controls.

The distribution of these trees was such that the distance between the two most widely separated trees was $\frac{5}{8}$ of a mile, and no two trees were more than $\frac{7}{16}$ths of a mile apart.

Times of spraying were :—

(a) Bud Movement (28–9–1946).

(b) After Bud Burst (14–10–1946).

(c) After Fruit Set (18–11–1946).

The days on which spraying was carried out were all fine and clear, with only light winds. The spray was applied between 10 a.m. and 4 p.m. Complete trees were sprayed, particular attention being given to the trunk.

The spray schedule is outlined in Table 5.

The disease first appeared on the control trees on the 28th November, 1946, while all four sprayed trees were completely free from disease. Data relating to the occurrence of the disease on the experimental trees is given in Table 6.

TABLE 6.

Tree No.	Spray.	Date.	Conditions of Tree. Remarks.
I.	Lime-sulphur .... Treatment with 2 sprays	28–11–1946	No trace of disease.
		2–1–1947	Traces of disease.
		16–2–1947	No further spread of disease on tree.
II.	Lime-sulphur Treatment with three sprays	28–11–1946	No trace of disease.
		2–1–1947	No trace of disease.
		16–2–1947	No trace of disease.
III.	Bordeaux mixture .... Treatment with 2 sprays	28–11–1946	No trace of disease.
		2–1–1947	Traces of disease.
		16–2–1947	Spread of disease on tree.
IV.*	Bordeaux mixture .... Treatment with three sprays	28–11–1946	No trace of disease.
		2–1–1947	Traces of disease.
		16–2–1947	No further spread of disease on tree.
V.	Control tree unsprayed	28–11–1946	Disease first apparent.
		2–1–1947	Disease well distributed over whole tree.
		16–2–1947	Disease much as on 2–1–1947 but defoliation had begun.
VI.	Control tree unsprayed	28–11–1946	Disease first apparent.
		2–1–1947	Disease distributed over whole tree, but particularly in one area.
		16–2–1947	Disease much as 2–1–1947, except that the area of tree which was badly infected at that time, now was practically devoid of foliage.

*This tree was within 20 yards of control tree V.

All trees used in the spraying experiments had been badly infected in the previous season, and it was assumed that all trees would have an approximately equal chance of further infection.

One control tree (V) was in the same garden as one sprayed tree (IV), the other trees were all solitary, and fairly evenly distributed.

This Spray Programme in all cases gave excellent control of the disease, apparently by destroying all overwintering inoculum held on the non-deciduous portions of the tree.

In this connection it may be noted that Masera, (6) advocates pruning of the young parts of the tree to reduce infection. This probably results in a reduction in overwintering spores, giving an effect similar to that of the spray treatment.

Removal and burning of all deciduous portions of the tree as soon as shed would remove the bulk of the overwintering inoculum before it had a chance to be dispersed, and so greatly reduce the potential infection source for the new season.

Of the sprays used, Lime-sulphur with three applications was the most effective ; and this, combined with the destruction of all deciduous portions, should go far towards the control of the disease.

ECONOMIC ASPECT.

As previously indicated mulberry trees have little economic value in this State. (In the 1945–46 season only 190 bushels, valued at approximately £950, passed through the markets). They are mainly grown in home gardens as a source of fresh fruit. In other countries, the mulberry tree is important because of its relation to the silk industry, and Masera (6) believes that the diseased leaves are toxic to silk worms. However, mulberry leaves sprayed with Lime-sulphur were found to be unpalatable to the silk worm (no record was made of the palatability of leaves sprayed with Bordeaux mixture).

It is apparent that sprayed trees retain their fruit load much better than the unsprayed control trees ; also, the sprayed trees appear to be almost free from " Bacterial Blight " as well as from the " leaf spot " disease. However, Bordeaux mixture left an undesirable residue on the fruit.

Estimations of the cost of applications of the sprays were made, and these were as follows :—

Lime-sulphur.—8s. 6d. approximately per tree for three sprays.

Lime-sulphur.—7s. approximately per tree for two sprays.

Bordeaux mixture.—3s. approximately per tree for three sprays.

These estimates include the calcium caseinate spreader.

ACKNOWLEDGMENTS.

The author is indebted to Mr. W. P. Cass-Smith, Government Plant Pathologist of the Department of Agriculture, W.A., for suggesting the subject for this investigation ; for advice during the whole investigation ; loan of apparatus and access to Departmental records, and also for criticism of the text. To Miss A. M. Baird and Miss O. Goss for valuable assistance and advice. Also to Mr. A. W. Brown, Mr. Dowell, Mr. J. Sherzinger and Mrs. M. R. Thomson who made their trees available for spraying.

REFERENCE TO LITERATURE.

1. *Ann. Rep. of Dept. of Agric. W.A.* (1943–44).
2. Clements and Shear, 1931, " Genera of the Fungi."
*3. G–C., J, 1927, " Maladies du Murier," R.A.M. VI., p. 567.
1927, *Rev. de Bot. Appliquee.* VII, 67, pp. 213–214.
4. Grove, W. B., 1937. " British Stem and Leaf Fungi." Vol. II.
*5. Ivanoff, 1926. "Cryptogamic Parasites of Cultivated Plants, Recorded in the last Five Years, (1921–1925)." *R.A.M.*, V. p. 519.
1926. *Agric. Inform. Period. Bull.* Sofia. VII, 3, pp. 14–17.
*6. Masera. 1933, " Observations on the Mulberry ' Scourge' " *Ann. Tecn. Agrar. Rome.* VI, 2, pp. 178–184. 1935. *R.A.M.* XIV., p. 265.
7. McAlpine D., 1895, " Systematic Arrangement of Fungi."
8. McLean and Cook. 1941. " Plant Science Formulae."
9. Rawlins, T. E., 1933. " Phytopathological and Botanical Research Methods."
10. Riker, A. J. and Riker, R. S., 1936. " Introduction to Research on Plant Diseases."
11. Stevens, 1925. " Plant Disease Fungi."
12. Tubeuf and Smith, 1897. " Diseases of Plants Induced by Cryptogamic Parasites."
*13. Wolf, F. A., 1935. "The Perfect Stage of a Leaf Spot Fungus on Red Mulberries." *J. Elisha Mitchell Sci. Soc.*, Vol. 51, pt. I. pp. 163–166. ; 1936 *R.A.M.* XV., p. 66.
*14. Zaprometoff. 1928. "Materials for Mycoflora of Central Asia." Pt. II. *Uzbekistan Exper. Plant. Prot. Stat.* Tashkent Publ. XI, iii, + 70 pp.
1929. *R.A.M.*, Vol. VIII, p. 338.
*15. Zaprometoff, and Mikhailoff. 1937. "Mulberry Diseases." *R.A.M.* XVI., p. 785. ; 1937. *Trans. Cent. Asian Sci. Res. Inst. Sericult. Tashkent.* XIV, 50 pp. (English Summary).

*Original references not available.